# *Mycobacterium tuberculosis* Meets the Cytosol: The Role of cGAS in Anti-mycobacterial Immunity

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**The intracellular fate of *Mycobacterium tuberculosis* is a subject of long debate. In this issue of *Cell Host & Microbe*, three independent studies reveal the detection of cytosolic mycobacterial DNA by the nucleotidyltransferase cGAS, emphasizing the concept of cytosolic access by *M. tuberculosis* and its role in balancing immune-protection and immune-pathogenesis.**

*Mycobacterium tuberculosis*, the etiological agent of human tuberculosis, is one of the most devastating pathogens in the history of mankind and continues to cause millions of active tuberculosis cases and more than a million deaths each year. This bacterium, which apparently evolved from a *Mycobacterium canettii*-like ancestor into an extremely widespread, aerosol-transmitted human pathogen (Boritsch et al., 2014), has developed the ability to circumvent many of the bactericidal mechanisms employed by the host. The interaction of *M. tuberculosis* with the host is highly complex, involving dedicated mycobacterial virulence factors as well as the action of the innate and adapted immune system, which is decisive for the control of infection or development of disease. A central role in this process is played by macrophages (Mϕ), which are the first to encounter the pathogen upon entry into the lungs, resulting in uptake and enclosure of *M. tuberculosis* in a phagosomal vacuole inside the Mϕ. While this initial cellular process is meant to eliminate phagocytized bacteria, *M. tuberculosis* has developed efficient strategies to prevent degradation and killing (reviewed in Majlessi et al., 2015 and Cambier et al., 2014).

In this context, three new independent studies in this issue of *Cell Host & Microbe* (Collins et al., 2015; Wassermann et al., 2015; Watson et al., 2015) explore the involvement of cyclic guanosine monophosphate-AMP (cyclic GMP-AMP, or cGAMP) synthase (cGAS) in sensing mycobacterial DNA and its cellular and immunological consequences, offering new insights into a fascinating chapter of mycobacterial host-pathogen research that has a long history of scientific excitement and critical discussion (reviewed in Majlessi et al., 2015).

In 1971, Armstrong and D'Arcy Hart first proposed that *M. tuberculosis* resists degradation in the phagosome by inhibiting fusion with lysosomes, favoring intraphagosomal survival and multiplication. Based on this landmark study and follow-up investigations, the paradigm emerged that intracellular *M. tuberculosis* resides and multiplies within a membrane-enclosed compartment, which is manipulated by the bacterium in a way that prevents maturation and acidification. Although this model of exclusive phagosomal containment is consistent with the production of dominant $CD4^+$ T cell responses specific to MHC-II-restricted epitopes derived from vacuole-resident microorganisms, the observed type I interferon (IFN) responses and activation of the cytosolic NLRP3 inflammasome suggest that *M. tuberculosis* also undergoes cytosolic stages during infection. While phagosomal egress of *M. tuberculosis* was evoked by several studies in the eighties and early nineties, including one by McDonough and colleagues in 1993, the subject remained debated (reviewed in Majlessi et al., 2015). A significant change in the perception of the field came about only more recently. First, a study by van der Wel and colleagues in 2007 used sophisticated cryo-electron microscopy to provide compelling evidence of apparent cytosolic presence of *M. tuberculosis* at later stages of infection. Then, in 2012, a collaborative study involving our group and that of J. Enninga demonstrated cytosolic access of *M. tuberculosis* in THP-1 human Mϕ by using a fluorescence resonance energy transfer (FRET)-based method. The latter approach relies on a change in the emission spectrum of a CCF-4 substrate due to β-lactamase-mediated cleavage of the substrate upon contact with *M. tuberculosis*. In both of these studies, cytosolic access of *M. tuberculosis* was dependent on the mycobacterial type VII secretion system ESX-1 and its secreted effectors, including the 6 kDa early secretory antigenic target ESAT-6 (also named EsxA), which has been shown to possess membranolytic activity. The ESX-1 system, which is absent from the live attenuated vaccine *Mycobacterium bovis* BCG due to the deletion of the region of difference 1 (RD1), is thus critical for cytosolic access of *M. tuberculosis* and the associated cell-biological parameters, which differ substantially between *M. tuberculosis* and BCG despite close genetic similarity (reviewed in Majlessi et al., 2015 and Boritsch et al., 2014). Finally, cytosolic access of *M. tuberculosis* is relevant not only in cultured phagocytes, but also in vivo, as recently demonstrated in an experimental mouse infection model that used a highly sensitive FRET-based detection method adapted for flow cytometry (Simeone et al., 2015). This study also showed that phagosomal membrane disruption occurred as early as 3 hours post-infection, consistent with the early kinetics of type I IFN production. However, the main contact of *M. tuberculosis* with the host cytosol built up over time to be at its maximum 2–3 days post-infection (Simeone et al., 2015), in agreement with aforementioned cryo-electron microscopy data.

From the comparison of ESX-1 proficient and deficient strains, it is clear

that cytosolic access has numerous consequences on the activation of different axes of innate immunity, among which the induction of type I IFN production may be considered as a pro-bacterial event, whereas the activation of the inflammasome and triggering of autophagy are thought to benefit the host. These opposing responses govern the balance between immune-pathogenesis and immune-protection.

The three new studies on the role of cGAS in anti-mycobacterial immunity now report on the cellular signaling events underlying this delicate balance. The nucleotidyltransferase cGAS is known to induce synthesis of type I IFN via cGAMP in response to cytosolic DNA derived from DNA viruses or HIV (reviewed in Cai et al., 2014). Similarly, cytosolic *M. tuberculosis* DNA is now found to induce the cGAMP signaling cascade in infected human and murine Mϕ. The detection of *M. tuberculosis* DNA by cGAS depends on ESX-1 and its secreted effectors, suggesting that signaling depends on pathogen-induced phagosomal rupture. The combined results support a model whereby subsequent to *M. tuberculosis* infection, cGAS and mycobacterial DNA co-localize in the Mϕ cytoplasm and form aggregates. This interaction initiates the synthesis of cGAMP by cGAS and consequent activation of the STING-TBK-1-IRF-3 signaling axis that drives transcription of IFN-β (Figure 1). cGAMP can also be transferred via gap junctions to adjacent bystander Mϕ, which will thus be efficiently activated without being infected (Wassermann et al., 2015).

However, within the host cytosol, mycobacterial DNA is also sensed by AIM-2, a sensor of the NLRP3 inflammasome complex that promotes the maturation of protective cytokines such as IL-1β. Interestingly, the cGAS-STING-TBK-1-IRF-3-IFN-I pro-bacterial axis and the AIM-2-NLRP3-IL-1β pro-host pathway were found to be exploited distinctly by different *M. tuberculosis* ESX-1 mutants (Wassermann et al., 2015). In THP-1 human Mϕ, ESX-1 deficient *M. tuberculosis* strains were unable to activate either of these two pathways, while certain *M. tuberculosis* secretion mutants (e.g., attenuated strain H37Ra or EspA/C transposon mutants), which express but do not secrete ESAT-6 (EsxA), continued to activate IL-1β, but not IFN-β production.

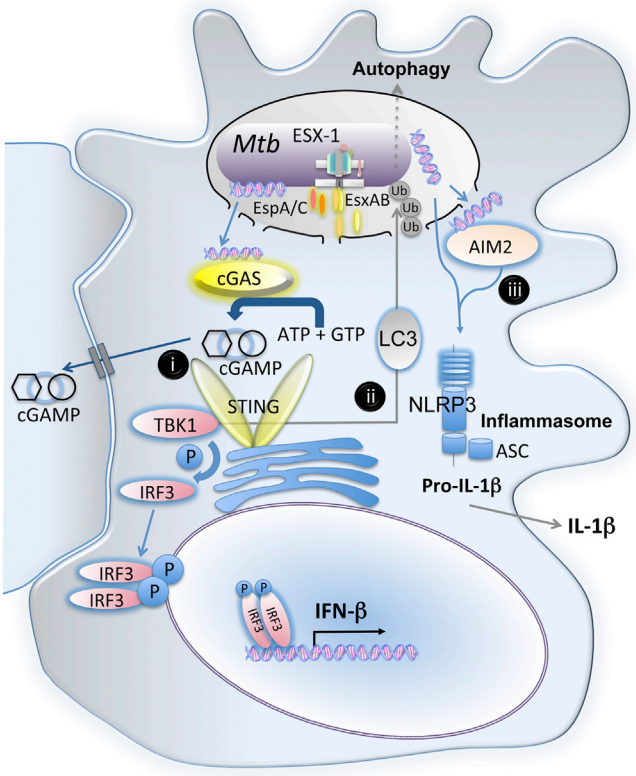


**Figure 1. Schematic of a Phagocyte Infected with *M. tuberculosis* and the Different Events Related to Cytosolic Translocation of Bacterial DNA**

The major mycobacterial virulence factor ESAT-6 (EsxA), secreted together with its protein partner EsxB and associated proteins EspA and EspC, by the type VII secretion system of *M. tuberculosis*, induce phagosome membrane rupture within the infected Mϕ. This event triggers cytosolic translocation of mycobacterial effector molecules such as DNA. (i) The latter is sensed by cGAS, which synthesizes the second messenger cGAMP from ATP and GTP. cGAMP activates the ER-associated STING and the downstream TBK-1-IRF-3-IFN-β signaling axis. cGAMP can also disseminate to bystander non-infected Mϕ to spread the cell activation. (ii) The ESX-1-mediated cytosolic translocation of bacterial DNA and the resultant activation of TBK-1 also initiate autophagy via recruitment of LC3-II involved in bacteria ubiquitination. (iii) The cytosolic bacterial DNA can also be sensed by AIM-2, which contributes partially to the activation of the inflammasome axis and release of mature IL-1β.

The ESX-1 system is also instrumental in mediating recruitment of the lipidated isoform of LC3 (LC3-II), which is associated with autophagosomes. This process is mediated via the cGAS recognition of mycobacterial DNA and is involved in ubiquitin-mediated activation of autophagy (Watson et al., 2015). However, ESX-1-dependent LC3-II recruitment has also been shown as being involved in the impairment of autophagic flux in human dendritic cells (Romagnoli et al., 2012), suggesting that the process is complex and possibly cell-type dependent.

Given the newly defined role of cGAS in anti-mycobacterial immunity derived from in vitro and ex vivo data, it will be interesting to learn more about the role of cGAS in vivo to know which of the pro-bacterial or pro-host-mediated roles emerges as being dominant there. Some insights come from one of the studies, where cGAS$^{-/-}$ mice challenged with virulent *M. tuberculosis* displayed reduced serum IFN-β levels, but increased lethality at late stages of infection, relative to wild-type mice, while the bacterial load was equal in both groups (Collins et al., 2015). More studies are warranted in the future, also to evaluate how mycobacterial antigen presentation and adaptive immunity are impacted in the absence of cGAS signaling.

Finally, one of the puzzling points to be considered is where the DNA that is sensed by cGAS comes from. Given that *M. tuberculosis* needs active secretion of ESX-1 effectors to gain access to the cytosol, mycobacterial DNA sensed by cGAS should thus originate from living bacteria and not from degraded ones. Possible explanations could be that *M. tuberculosis* buds off vesicles that contain mycobacterial DNA (reviewed in Majlessi et al., 2015) or that the bacterium releases DNA by other means, such as the

type VII secretion system. More research is also warranted here.

Together, the results shown in the three articles present exciting new insights into the tuberculosis-related host-pathogen interaction and raise many new questions and challenges for future work on *M. tuberculosis* pathogenicity and vaccine design (Bottai et al., 2015). The molecular details on the mechanisms through which *M. tuberculosis* gains access to the host cytosol and the biological consequences remain of central importance.

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